Deep impact: in situ functional responses reveal context-dependent interactions between vertically migrating invasive and native mesopredators and shared prey

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SUMMARY
1. The ecological effects of invasive species depend on myriad environmental contexts, rendering understanding problematic. Functional responses provide a means to quantify resource use by consumers over short timescales and could therefore provide insight into how the effects of invasive species vary over space and time. Here, we use novel in situ microcosm experiments to track changes in the functional responses of two aquatic mesopredators, one native and the other an invader, as they undergo diel vertical migrations through a lake water column.
2. The Ponto–Caspian mysid, Hemimysis anomala, a known ecologically damaging invader, generally had higher a functional response towards cladoceran prey than did a native trophic analogue, Mysis salmaai. However, this differential was spatiotemporally dependent, being minimal during the day on the lake bottom, and increasing at night, particularly inshore.
3. Because the functional response of the native predator was spatiotemporally consistent, the above pattern was driven by changes in the invader functional response over the diel cycle. In particular, the functional response of H. anomala was significantly reduced on the lake bottom during the daytime relative to night, and predation was especially pronounced in shallow surface waters.
4. We demonstrate the context dependency of the effects of an invasive predator on prey populations and emphasise the utility of functional responses as tools to inform our understanding of predator–prey interactions. In situ manipulations integrate experimental rigour with field relevance and have the potential to reveal how impacts manifest over a range of spatiotemporal scales.

Keywords: diurnal vertical migration, ecological impacts, functional response, in situ predation, invasive species

Introduction
Understanding how invasive species affect recipient communities remains a focal issue in ecology, yet there is a prevailing perception that such understanding will remain elusive (Gilpin, 1990; Williamson, 1999; Simberloff et al., 2013), primarily because impacts are often strongly context dependent (Ricciardi et al., 2013). Impacts may vary over space and time, where effects are modified by myriad environmental factors, both abiotic (e.g. temperature) and biotic (e.g. predator interference) (Barrios-O’Neill et al., 2014; Dick et al., 2014). In addition, the scale at which effects are considered will have a strong bearing on conclusions about their direction and magnitude, as is the case with diversity–invasibility relationships (Byers & Noonburg, 2003). For example, the effects of invasive species can change dramatically over decadal timescales (Strayer et al., 2006). Little is known, however, about how effects may vary over much shorter timescales, despite the fact that seasonal heterogeneity and diurnal heterogeneity are fundamental to the structure and function of ecological communities, and are well known to mediate species interactions (Townsend & Risebrow, 1982; Viherluoto & Viitasalo, 2001). Spatial
variations in effects are better understood at finer scales and can be linked to spatial heterogeneity, whether exogenous, that is, mediated by variation in abiotic conditions (Kestreup, Dick & Ricciardi, 2010), or endogenous, that is, mediated by the interactions and dispersal of species (Melbourne et al., 2007). Even so, finely resolved data on spatial impacts are scarce, because many inferences about the impacts of invasive species are drawn from raw observations of range size and abundance (Ricciardi et al., 2013).

Despite the complexities of biological invasions, efforts to understand their impacts have revealed some useful generalisations (Ricciardi et al., 2013). For example, the invasion history of a species can be a useful indicator of its potential impacts elsewhere (Grosholz & Ruiz, 1996; Kulhanek, Ricciardi & Leung, 2011), but this approach intrinsically lacks relevance to novel invaders. In contrast, certain ecological traits can be linked to invasiveness and perhaps also to impact (e.g. Pyšek et al., 2012), thus providing a means by which to anticipate the effects of invaders a priori. However, trait-based approaches that rely solely on correlates between impact and the attributes of successful invaders may be confounded because they make no provision for how invaders interact with the recipient environment (Ricciardi et al., 2013). Therefore, methodologies that have the capacity to unify species traits with specific environmental context dependencies could advance our understanding of impact.

One promising trait-based methodology that can incorporate environmental context involves consideration of the relationship between resource use by invaders and their associated ecological impacts. Analysis of consumer functional responses (i.e. resource usage in relation to resource availability) provides a robust means to understand the stability of prey populations (Kalikat et al., 2013) and food webs (Williams & Martinez, 2004) and has a range of potential applications in invasion ecology (Dick et al., 2014). A fundamental advantage of functional responses is their capacity to quantify the density dependence of the relationship between resource availability and usage, which avoids the pitfalls of snapshot assessments of resource usage (Dick et al., 2014). Because functional responses underpin many concepts in ecology (e.g. Rall et al., 2012), it is perhaps surprising that they have only recently been recognised as useful tools by invasion ecologists (Dick et al., 2013; Barrios-O’Neill et al., 2014). It is particularly apparent that functional responses could inform impact assessment frameworks sensu Parker et al. (1999), because they provide a rapid, tractable means of estimating the per capita effects of invaders, a fact which has not gone unnoticed in biological control research (Zamani et al., 2006). Moreover, functional responses can provide alternative insights into well-established hypotheses in the field by, for example, quantifying biotic resistance (MacNeil et al., 2013).

The methods whereby functional responses are typically derived may provide only rudimentary insights into the context dependency of impact. Field-derived functional responses may intrinsically capture multiple context dependencies, but lack sufficient resolution of shape at low resource densities, which is crucial to mediating the stability of consumer-resource dynamics (Williams & Martinez, 2004; Kalinkat et al., 2013). Furthermore, field-derived functional responses often combine data from a range of locations (e.g. Angerbjorn, Tannerfeldt & Erlinge, 1999), with the implicit assumption that functional responses are consistent over space and time. In contrast, laboratory-derived functional responses can provide such resolution and quantify specific context dependencies (e.g. Kestreup et al., 2010), but may lack ecological realism (Aljetlawi, Sparrevik & Leonardsson, 2004). Thus, understanding whether and how functional responses vary spatio-temporally, both within and between habitats, remains a considerable challenge in contemporary ecology.

Here, we address this knowledge gap using in situ experimental microcosms to compare the functional responses of two aquatic invertebrate predators, both mysid shrimps, that have invaded habitats outside their native ranges: the Ponto–Caspian Hemimysis anomala Sars, 1907 and the glacial relict Mysis salemaai Audžijonytė and Väinölä, 2005. In Ireland, H. anomala has established within the native range of M. salemaai, where restricted waterbody size results in their co-occurrence (Minchin & Boelens, 2010). Previous laboratory studies demonstrated that H. anomala has consistently higher functional responses towards several prey species than does M. salemaai (Dick et al., 2013; Barrios-O’Neill et al., 2014). Both H. anomala and M. salemaai undergo diel vertical migration (DVM), which is a variable phenomenon, but generally involves aggregation close to the bottom during the daytime followed by dispersal upward towards the surface at night (Hays, 2003). We sought to understand how the ecological effects of these mysids manifest in the waterbodies they inhabit, by conducting in situ functional response trials designed to reflect realistic spatiotemporal distributions over their DVM cycles. We used Daphnia magna Straus, 1820 as a representative prey species because cladocerans are frequently adversely affected by introductions of mysids (Goldman et al., 1979; Ketelaars et al., 1999), and because cladocerans also undergo DVM (Southern & Gardiner, 1932). By transferring a rigorous laboratory-based methodology
into the field, we aimed to combine improved ecological realism with the capacity to adequately resolve functional response shape and, thus, explore the potential ecological effects of these predators on prey populations.

Methods

Animal collection and study site

During August 2012, we collected *Hemimysis anomala* and *Mysis salenai* (wet weight ± SE = 18.6 mg ± 0.1 and 19.2 mg ± 0.2, respectively) from Lough Neagh, County Derry, Northern Ireland (54.71000°N, 6.49017°W) and Lough Derg, County Tipperary, Republic of Ireland (52.90556°N, 8.34158°W and 52.90782°N, 8.35553°W) using a combination of horizontal and vertical tows with a plankton net (1 m mouth diameter, 1 mm mesh size). Lough Derg is a mesotrophic lake with a maximum depth of 36 m which forms part of the Shannon River system. We selected this lough to conduct our *in situ* study because the deepest parts are close to the shore, and because the distributions of *H. anomala* and *M. salenai* overlap on the lough (Minchin & Boelens, 2010).

Samples were separated by species and transferred into 50 L covered opaque holding tanks situated at a shoreside location on Lough Derg (52.90523°N, 8.34507°W). *Daphnia magna* was used as prey in all trials, maintained in culture at Queen’s University Belfast and transported in identical holding tanks to this shoreside location. Each holding tank was filled with 40 L of filtered lough water and containing *D. magna* prey at one of seven densities (2, 4, 6, 8, 15, 25 and 40, *n* = 3 each). Controls with no predators consisted of densities 2, 15 and 40 (*n* = 3 each). Predator–prey combinations and controls were randomly distributed on arrays, and all chambers were sealed with lids before deployment. Arrays were subsequently deployed within 15 min of the initiation of trials at five ‘locations’ (here, location refers to both time and space) reflecting a typical DVM cycle over 24 h (i.e. distributed through the water column during night and aggregated close to the bottom during daytime) (Fig. 1). Thus, locations for functional response trials were as follows: on the bottom during daytime at the shallow and deep sites (3 m and 20 m, respectively) and, during night, on the surface (1 m) at both sites, as well as on the bottom at the deep site. The latter location reflects the fact that mysids still occur in deeper water during the night, despite a general migration towards the surface (Southern & Gardiner, 1932).

Trials were terminated after 6 h on retrieval of the arrays and removal of predators, after which surviving *D. magna* were counted.

Statistical methods

All statistical analyses were undertaken using R (R Development Core Team, 2013). Generalised linear models (GLMs) were used to assess the consumption of practically possible, both predators and prey were exposed to ambient cues, particularly because non-lethal cues consistently invoke antipredator responses in aquatic invertebrates (Paterson *et al.*, 2013). Therefore, all trials were conducted in purpose-built arrays constructed from clear polycarbonate sheeting, supporting grids of 50 × 200 mL clear plastic containers (hereafter chambers), into which 6 cm diameter windows were cut and sealed with 40 μm nylon mesh and non-toxic clear aquatic silicone (Fig. 1). Previous laboratory studies have demonstrated that meshed chambers of this kind allow the transmission of ambient cues to predators and prey (e.g. Alexander, Dick & O’Connor, 2013). Each array was deployed vertically on a buoyed line during experimental trials. Two sites on Lough Derg were selected to deploy arrays (Fig. 1); a shallow site (site 1, 52.90556°N, 8.34158°W, 4 m deep) and a deep site close to the deepest part of the lough (site 2, 52.90782°N, 8.35553°W, 22 m deep). Prior to deployment, arrays were prepared at the shoreside location; trials were initiated on the introduction of single mysid predators into chambers filled with 40 μm filtered lough water and containing *D. magna* prey at one of seven densities (2, 4, 6, 8, 15, 25 and 40, *n* = 3 each). Controls with no predators were as follows: on the bottom during daytime at the shallow and deep sites (3 m and 20 m, respectively) and, during night, on the surface (1 m) at both sites, as well as on the bottom at the deep site. The latter location reflects the fact that mysids still occur in deeper water during the night, despite a general migration towards the surface (Southern & Gardiner, 1932).

Trials were terminated after 6 h on retrieval of the arrays and removal of predators, after which surviving *D. magna* were counted.

**In situ functional responses: experimental design**

We sought to derive *in situ* functional responses for this mysid–cladoceran study system where, as far as
Consumption of Daphnia magna by mysid predators among all array locations. Then, after using logistic regression to determine the appropriate functional response types (see Juliano, 2001), we fitted functional response curves using maximum likelihood estimation (MLE; Bolker, 2010). Nonparametric bootstrapping was used to construct 95% confidence intervals around functional response curves and their associated parameters (Pritchard, 2013).

Functional responses of mysid predators

To fit appropriate functional response models to the data, we first used logistic regressions to determine the shapes of the relationships between proportional prey consumption and prey density. Where proportional consumption declines with increasing prey density, the logistic regression yields a significant negative first-order term, and the functional response is appropriately described by a Type II hyperbola. Proportional consumption which is described by a significant positive first-order term followed by a significant negative second-order term indicates that the functional response is appropriately described by a sigmoidal Type III model (Juliano, 2001). Because the logistic regressions for both GLMs which contained the factors ‘array location’ (five levels: Fig. 1), ‘prey density’ (seven levels) and their associated interaction were simplified stepwise using F tests. If ‘array location’ was required by the minimum adequate model, then post hoc comparisons of linear coefficients with Tukey’s HSD method were used to identify specific differences.

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predators at all locations yielded negative first-order terms (Table 1), functional responses were subsequently modelled using MLE with the random predator equation, which assumes a Type II shape and accounts for the non-replacement of prey as they are consumed (Rogers, 1972):

\[ N_e = N_0(1 - \exp(\alpha N_0 h - T)) \]  

(1)

where \( N_e \) is the number of prey eaten, \( N_0 \) is the initial density of prey, \( \alpha \) is the attack or capture rate, \( h \) is the handling time and \( T \) is the total time available.

To construct 95% confidence intervals around functional response curves and their associated parameters, data sets were nonparametrically bootstrapped (\( n = 2000 \)) and eqn 1 was fitted to each bootstrapped data set using starting values of \( \alpha \) and \( h \) from the original MLE. Because bootstrapping allows data to be considered in terms of populations, as opposed to samples, a lack of overlap between 95% confidence intervals is sufficient to ascribe significance to differences between treatments without recourse to parametric tests. Therefore, we present both functional responses and their associated model parameters graphically with 95% confidence intervals to quantify differences between invaders and natives at the same array location, and also for invaders and natives among all array locations.

**Results**

Control *Daphnia magna* survival was 100% after 6 h at all control densities, at both sites and at all locations; therefore, we deemed all experimental mortality to be a result of mysid predation. Although predation was impossible to observe directly, it was also evidenced by the partial remains of prey.

### Consumption of Daphnia magna by mysid predators

*Hemimysis anomala* consumed significantly more *Daphnia magna* than did *Mysis salemaai* at all locations during the night (see Fig. 2a–c): at the shallow site on the surface (\( t = 5.10, P < 0.001 \)), at the deep site on the surface (\( t = 2.43, P = 0.015 \)) and at the deep site on the bottom (\( t = 3.60, P < 0.001 \)). In contrast, on the bottom during the day (see Fig. 3a,b), there were no significant differences in consumption of *D. magna* by *H. anomala* and *M. salemaai* at either shallow (\( t = 0.89, \text{NS} \)) or deep (\( t = 1.82, \text{NS} \)) sites.

Notably, predation by *M. salemaai* was consistent among all locations because the factor ‘array location’ could be removed from the minimum GLM (\( F_{4, 103} = 2.03, \text{NS} \)). In contrast, consumption of *D. magna* by *H. anomala* was markedly asymmetric among locations, because the factor ‘array location’ was required in the minimum GLM (\( F_{4, 103} = 7.67, P < 0.001 \)). Differences were driven by a relative reduction in feeding by *H. anomala* at both sites on the bottom during daytime (Fig. 2a–c with Fig. 3a,b). In particular, consumption was lower on the bottom at the shallow site during daytime than at all locations during the night, at the deep site both on the bottom (\( P < 0.001 \)) and on the surface (\( P = 0.002 \)), and at the shallow site on the surface (\( P < 0.001 \)). Consumption was also lower at the deep site on the bottom during daytime than at the shallow site on the surface during the night (\( P = 0.031 \)).

### Functional responses of mysid predators

*Hemimysis anomala* had higher functional responses towards *Daphnia magna* than did *Mysis salemaai* at all locations during the night: at the shallow site on the surface (Table 1).
surface (Fig. 2a), at the deep site on the surface (Fig. 2b) and at the deep site on the bottom (Fig. 2c). Although predator functional responses were similar at both sites on the bottom during the day (Fig. 3a,b), bootstrapped 95% confidence intervals revealed more subtle differences. Specifically, confidence intervals were divergent between invader and native functional responses for the entire range of prey densities only at the shallow site on the surface during the night (Fig. 2a). In contrast, invasive and native functional responses were not significantly different at the same site on the bottom during the day, as 95% confidence intervals overlapped throughout (Fig. 3a). At all other locations, the overlap of native and invasive functional responses was dependent on prey density (Figs 2b,c and 3b).

Bootstrapped estimates of attack rates and handling times corroborated the lack of differences and hence observed consistency of predatory activity by *M. salmaai*, because 95% confidence intervals overlapped among all locations, both for attack rates (Fig. 4a–e, filled circles) and handling times (Fig. 4f–j, filled circles), although handling times were longest on the surface at the shallow site (Fig. 4f, filled circle). On the other hand, estimates of attack rates and handling times for *H. anomala* were more variable among locations. Attack rates were lower at the shallow site on the bottom during the day than at all locations during the night (Fig. 4d and a–c, respectively, open circles), whilst handling times were longer at the deep site on the bottom during the day than at all locations during the night (Fig. 4j,f–h, respectively, open circles). The attack rates of invasive

![Fig. 2](image1.png)  ![Fig. 3](image2.png)

**Fig. 2** Functional responses of *Hemimysis anomala* (dashed lines) and *Mysis salemaai* (solid lines) towards *Daphnia magna* at three locations during the night: (a) 1 m at the shallow site, (b) 1 m at the deep site and (c) 20 m at the deep site. Shaded areas are bootstrapped 95% confidence intervals.

**Fig. 3** Functional responses of *Hemimysis anomala* (dashed lines) and *Mysis salemaai* (solid lines) towards *Daphnia magna* at two locations during the day: (a) 3 m at the shallow site and (b) 20 m at the deep site. Shaded areas are bootstrapped 95% confidence intervals.

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and native mysids exhibited a degree of overlap at each location (Fig. 4a–e); thus, observed differences in functional responses were driven primarily by the shorter handling times of the invader during the night (Fig. 4f–h). Although the handling times of the invader exhibited some degree of overlap with those of the native during night, they were consistently shorter, significantly so inshore on the surface (Fig. 4f).

**Discussion**

We demonstrate that the invasive mysid *Hemimysis anomala* has generally higher functional responses over its diurnal vertical migration (DVM) cycle than does the native species *Mysis salemaai*, corroborating previous laboratory findings (Dick et al., 2013; Barrios-O’Neill et al., 2014). Crucially, however, we highlight the context dependency of these functional responses; the impacts of *H. anomala* were particularly asymmetric over its DVM cycle, with a marked reduction in feeding on the bottom during daytime and higher feeding during the night, particularly at the shallow site. In contrast, *M. salemaai* fed consistently over its DVM cycle, demonstrating the higher relative importance of daytime predatory activity for this species. Further, the broad consistency of evidence for the ecological effects of *H. anomala* between laboratory manipulations (Dick et al., 2013; Barrios-O’Neill et al., 2014), field manipulations and field surveys (Ketelaars et al., 1999) alludes to the potential value of functional responses as a tool to inform assessments of impact.

In large waterbodies, *H. anomala* is essentially littoral, being found at a maximum depth of 50 m during the daytime (but frequently much shallower), whilst *Mysis* spp. generally occur further offshore (Ricciardi, Avlijas & Marty, 2011). However, in Irish waterbodies where *M. salemaai* is native and *H. anomala* is an established invader, maximum depths rarely exceed 30 m, resulting in a high degree of spatial overlap between these species (Minchin & Boelens, 2010). Emerging evidence for the seasonally mediated presence of *M. salemaai* in the littoral zone of some lakes (Penk & Minchin, 2014), combined with evidence of profundal aggregations of *H. anomala* (Ketelaars et al., 1999), highlights the need to better understand the consequences of interactions of these species – with each other and with prey. Nevertheless, even in relatively small waterbodies such as Lough Derg, *H. anomala* is more prevalent in the shallows, whilst *M. salemaai* is common in deeper areas. Thus, we may speculate that observed differences in predatory behaviour manifest primarily because of the typical distributions of these species in larger systems; in the case of the invader, daytime aggregation in the littoral zone may necessitate reduced activity because its visually orientated potential predators may be more effective in well-lit conditions (Hays, 2003). Conversely, where native mysids have the available depth, daytime migrations into deep water may result in a perpetual refuge effect, where the abundance and/or efficacy of its predators is continuously limited. Therefore, where these species co-occur as a result of waterbody size and morphology, endogenous behaviour may determine how their impacts manifest. Indeed, although there is some debate as to the extent to which mysids rely on vision to capture prey (Viherluoto & Viitasalo, 2001), predator avoidance behaviour may ultimately dictate maximal feeding (Paterson et al., 2013; Barrios-O’Neill et al., 2014), regardless of ambient light. Notably, Barrios-O’Neill et al. (2014) found evidence for predator avoidance behaviour in *M. salemaai* but not *H. anomala* during low-light trials. These findings and those of the current
The shape and magnitude of predator–prey interactions are considered crucial determinants of population and food-web stability, resulting in the incorporation of functional responses into contemporary modelling approaches (Williams & Martínez, 2004; Vucic-Pestic et al., 2010; Kalinkat et al., 2013). But we know very little about how functional responses vary in time and space under different environmental conditions, in spite of the well-recognised dynamic nature and context dependency of species interactions (Powers & Kittinger, 2002; Leahy et al., 2011). Laboratory studies may yield broad insight into how, for example, light intensity (Townsend & Risebrow, 1982) impinges on functional response shape and magnitude, but it is also apparent that predator–prey dynamics hinge on very small changes in the functional response (Williams & Martínez, 2004). Altogether, the value of an in situ approach is evident, particularly when considering the sensitivity of predator–prey dynamics in conjunction with the fact that natural systems are replete with cues that shape the outcomes of interactions and prey fitness (Orrock et al., 2013; Paterson et al., 2013). Conceptually, predator–prey interactions can be thought to map over habitats at various spatiotemporal scales, and therefore resolving interactions at the appropriate scale may inform our understanding of the persistence and stability of food webs. This point is particularly relevant to risk assessments concerned with how novel species will affect recipient communities, because spatial and temporal changes in interactions and subsequent impacts are characteristic of invasions (Byers & Noonburg, 2003; Strayer et al., 2006).

It is clear that not all study systems will be practically amenable to in situ manipulations. Nevertheless, the scope for application remains broad, and functional responses are not specific to predator–prey systems (e.g. they are well demonstrated in herbivores; Gross et al., 1993). Further, although the present study highlights functional response dynamics at a diurnal scale, there is clearly scope within this mysid study system to address questions relating to seasonal (Penk & Minchin, 2014), ontogenetic, allometric (Boscaino et al., 2012) and prey-specific (Dick et al., 2013) interaction patterns. Broadly, then, the range of potential applications across invasion science as a whole is likely to be similarly diverse. Additionally, functional responses are relatively rapidly derived, thus providing a tractable proxy to inform assessments of impact. There is a clear need for proxies of this kind, as limited resources are available to study and manage the burgeoning number of invasions, and there is growing pressure to prioritise (Hulme et al., 2013). Managers and researchers alike require reliable methods with which to assess impact without recourse to extensive, time-consuming analysis of entire ecosystems.


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